# AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS ISOLATED FROM EPIDEMIC POLIOMYELITIS BY IMMUNE SERUM

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Recent investigations on the bacteriology of poliomyelitis have shown quite constantly in the atria of infection and in the infected tissues of epidemic poliomyelitis a pleomorphic streptococcus or micrococcus which, soon after isolation, has tended to localize electively in the central nervous system of animals and to produce paralysis. These properties are soon lost on cultivation. The importance of immunologic studies was recognized early and Sept. 11, 1916, immunization experiments were begun by the injection of a monkey with the pleomorphic streptococcus in order to protect it against virus should it recover from the effects of the injection.[1] Since then, horses and monkeys have been immunized and their serum tested for immune bodies (chiefly agglutinins), for neutralizing and protecting power over virus, and for curative effects on experimental poliomyelitis in monkeys. The agglutinating power of the serum of patients and monkeys that have recovered from poliomyelitis, together with numerous normal controls, and its curative effect on poliomyelitis in man has been studied. Summaries of these studies have already been published.[2, 3] In this paper we wish to record in greater detail the experiments and results obtained.

## TECHNIC

In the immunization of horses increasing doses of the pleomorphic streptococcus were injected intravenously on 3 consecutive days, with an interval of a week between each series from November 2 to May 1. The bacteria used for the injections were grown in dextrose broth or ascites dextrose broth for 24 hours, then centrifugalized out and suspended in salt solution.

Horse 1 was injected from Nov. 2, 1916, to May 1, 1917. At first strains from human poliomyelitis (heated to 60 C.) were injected, and for a short time both human and monkey strains were used. For many weeks, however, live cultures of strains from experimental poliomyelitis in monkeys, were injected exclusively. Test bleedings were made November 2, 4 and 22, December 22, January 8 and 30, March 3, April 3, and May 14 and 16. The serum obtained

December 22, 7 weeks after immunization was begun, agglutinated the pleomorphic streptococcus in dilutions of 1:6150 and showed neutralizing and protecting power over virus.

Horse 2 was injected from November 22 to December 13 with live cultures from human and monkey poliomyelitis. During the injection, December 13, the animal died from acute anaphylactic shock. It was bled before the injection, and soon after death.

Horse 3 was injected from January 30 to May 12 with strains from human poliomyelitis. Test bleedings were made January 30, March 3, April 3, and May 14.

There was difficulty in preparing satisfactory antigens for agglutination purposes. The pleomorphic streptococcus, as pointed out in the preliminary report,[3] tends to grow in clumps or to clump spontaneously in salt solution suspensions. In attempting to overcome this difficulty the character of growths in various liquid mediums was studied under various conditions, and it was found that after incubating from 33-35 C., tall columns of dextrose broth or ascites dextrose broth (0.2% dextrose, 0.6% acid to phenolphthalein, 10% ascites fluid) growth was usually diffuse and marked at the end of from 16-24 hours.

By now neutralizing the broth to phenolphthalein with sodium hydroxid and placing it in the ice chest, or by centrifugalizing out the bacteria and making dense suspensions in normal salt solution the difficulty was largely overcome. The common 6 ounce nursing bottle filled to a depth of 9.5 cm. containing 150 c.c. of broth was found most satisfactory. The tendency to clump-formation was, for unknown reasons, less in these containers than in test tubes containing the same medium, even in columns equally tall. Growth almost invariably began in the deeper layers and would reach the top first, after the growth pressure was at its maximum. Centrifugalization or neutralization was usually done when the growth had extended to within 1-0.5 cm. of the top. The suspensions used as stock solutions were made so that 1 c.c. of the suspension contained the bacteria from 15 c.c. of the broth culture. The antigens in the experiments, unless otherwise mentioned, consisted of the stock suspensions diluted with salt solution immediately before using to the density of the broth culture. The ascites dextrose and dextrose-broth cultures, while fresh, were almost as satisfactory as salt solution suspensions, but spontaneous clumping usually occurred even after neutralization in from a few days to a week or occasionally longer. The bacteria remained in a suitable condition for agglutination tests for a long time in the salt solution suspensions when kept in the ice chest. This was an important point for the specific agglutinating property tends to be lost on artificial cultivation especially aerobic cultivation. Equal parts (usually 0.2 c.c. each) of bacterial suspension or antigen and serum or dilution of serum were thoroughly mixed. Two types of dilutions of serum were used: Progressive 1:5, and progressive 1:10 dilutions, beginning with equal parts of serum and antigen. The mixtures were incubated for 1½ hours. In the earlier part of the work a reading was made and the tubes placed in the ice chest over night after which a second reading was made. The latter was found to be more reliable and was ultimately adopted. The readings were made against a black background through intense transmitted light from a 100 watt nitrogen bulb so shaded as to protect the eyes. The degree of agglutination is indicated in the tables by 1 or more plus (+) signs, no agglutination by 0. One plus sign

Fig. 1.—Photomicrograph of a hanging drop of mixture of a suspension of the pleomorphic streptococcus (M 49.4) and normal monkey serum (M 121) diluted 1: 100. Note the even distribution of the bacteria. This mixture had been incubated 1½ hours and then kept in the ice chest over night (× 200).

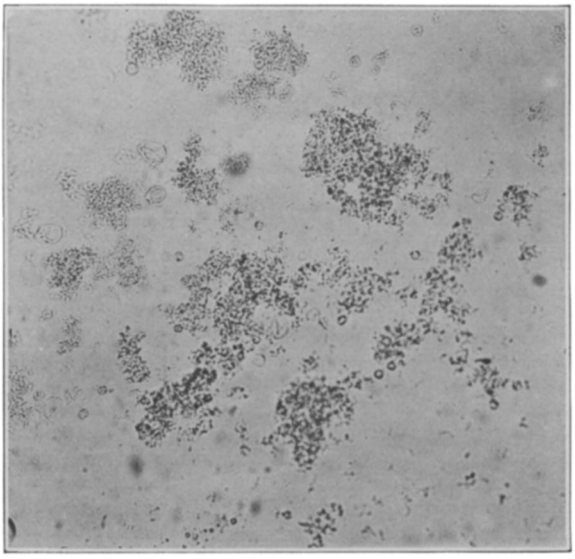

Fig. 2.—Same as Fig. 1, but with the serum of monkey (M 105) paralyzed with virus. Note the marked agglutination of the bacteria. The reading of result in case of Fig. 1 in the macroscopic test was 0, in Fig. 2 ++ (× 200).

indicates an undoubted clumping, 2 plus signs clumping with some clearing of the mixtures, 3 plus signs marked clumping with complete clearing of the mixtures, and 4 plus signs the same as 3 except that the clumps were larger. The value of the signs is well illustrated in Figure 3. At first agglutination was studied in hanging drop, to make sure that what appeared as macroscopic agglutination meant a real clumping of the streptococci (Figs. 1 and 2). In the low dilutions of the immune serum and antigen there was often an increased clouding of the mixture with or without precipitation. This is indicated in the tables by a "C" affixed to the sign of agglutination. Parallel tests were made with the serum of normal animals corresponding to the species from which the

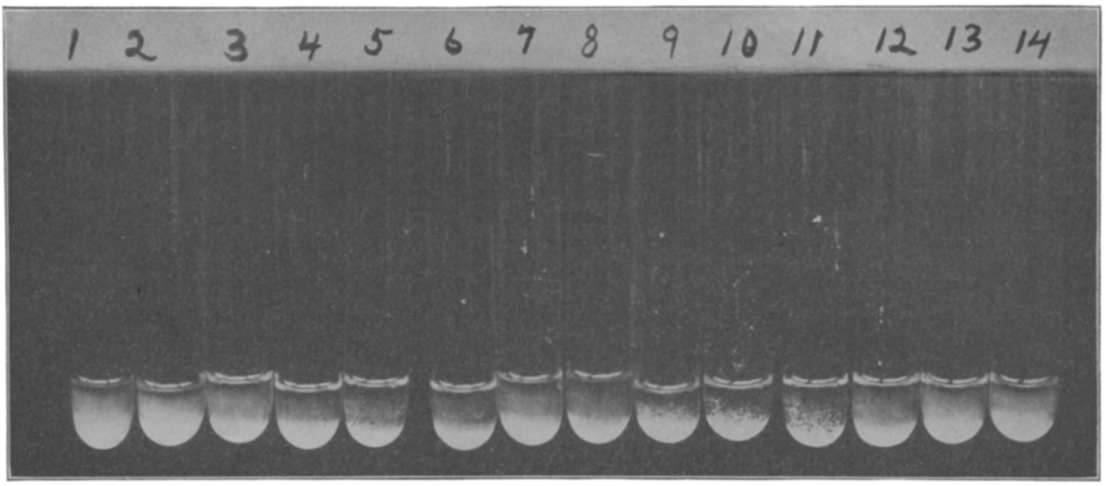


Fig. 3.—Agglutination of human (721.4².2) strain by the serum of Horse 1 (Tubes 1-7) and of monkey strain (M 49.4) with the serum of Horse 3 (Tubes 8-14). The first tube in each series contained equal parts of antigen, normal salt solution, suspensions of the bacteria, and serum; the others contained equal parts of antigen and progressive 1: 10 dilutions of serum in normal salt solution. Note the increasing agglutinating power of these serums with increasing dilutions up to 1: 10,000 and its rapid disappearance in dilutions above this point, there being slight agglutination in 1: 100,000 dilutions but none in 1: 1,000,000 dilutions.

immune serum was obtained. Controls in normal salt solution and control strains of streptococci were included and in addition the agglutinating power of antipneumococcus and antistreptococcus serum over the pleomorphic streptococcus was tested. In the text and tables, for the sake of simplicity, the exponent affixed to a given number of animal or strain indicates animal passage whereas the figure after the period indicates the culture generation; thus 714².3 indicates that this strain has been passed through one animal and that it is in the third culture generation. For the same reason the expressions "human" and "monkey" poliomyelitis are used. "Virus immune" monkeys are those injected with emulsions or filtrates of poliomyelitic brain and cord and which developed typical poliomyelitis; "culture immunes" are those which were injected with cultures of the pleomorphic organism.

## AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS BY THE SERUM FROM HORSES

In Table 1 is given the agglutinating titer of the serum from Horse 1 (immunized chiefly with monkey strains), against a strain from human poliomyelitis. The strain used in this experiment was isolated a short time previously from the dried brain of a patient (Case 714). The agglutinating power of this serum before injection and 2 days after the first injection corresponds quite closely to what has been observed in the serum of normal horses. The increase in agglutinating power of the serum in the successive bleedings is well shown. Similar results were obtained with other strains and with the serum from Horse 2 and Horse 3. The agglutinating titer of the

TABLE 1

AGGLUTINATING TITER OF THE SERUM OF VARIOUS BLEEDINGS FROM HORSE 1 OVER THE PLEOMORPHIC STREPTOCOCCUS (714) FROM THE BRAIN IN HUMAN POLIOMYELITIS

| Dilutions of Serum | Serum from Horse 1 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nov. 2 | Nov. 4 | Nov. 22 | Dec. 22 | Jan. 8 | Jan. 30 | March 3 |
| 1 : 1 | +++ | +++ | +++ | + | +c | +c | ++c |
| 1 : 10 | ++ | ++ | ++++ | ++ | ++ | ++ | +++c |
| 1 : 50 | 0 | 0 | ++ | ++++ | ++++ | ++++ | ++++ |
| 1 : 250 | 0 | 0 | ++ | +++ | ++++ | ++++ | +++ |
| 1 : 1250 | 0 | 0 | 0 | + | + | +++ | + |
| 1 : 6150 | 0 | 0 | 0 | + | 0 | ++ | + |
| 1 : 30,250 | 0 | 0 | 0 | 0 | 0 | 0 | + |

TABLE 2

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS FROM HUMAN POLIOMYELITIS (714) UNDER VARIOUS CONDITIONS

| Serum from | Dilutions of Serum | Pleomorphic Streptococcus (714) in | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dextrose Broth Culture | | Suspension of NaCl Solution | | | | | |
| | | | | Of the Density of the Broth Culture | | | | | 15 Times the Density of the Broth Culture |
| | | Acidity of 48 Hour Culture | Neutralized to Phenolphthalein | Untreated | Heated to 60 C. 30 Min. | Heated to 100 C. 3 Min. | + .5% Formalin | + .5% Phenol. | |
| Normal Horse | 1 : 1 | +++ | ++ | +++ | +++ | +++ | + | +++ | ++ |
| | 1 : 10 | ++ | + | ++ | ++ | +++ | + | ++ | + |
| | 1 : 50 | 0 | 0 | + | 0 | ++ | + | 0 | 0 |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Horse 1 (Jan.30) | 1 : 1 | + | 0 | + | ++ | ++ | + | + | +++ |
| | 1 : 10 | +++ | ++ | +++ | +++ | +++ | ++++ | ++ | ++++ |
| | 1 : 50 | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++++ |
| | 1 : 250 | +++ | ++++ | ++++ | +++ | +++ | +++ | ++ | ++++ |
| | 1 : 1250 | +++ | +++ | +++ | + | +++ | ? | + | + |

serums on April 3 was no higher than on March 3. It is of interest to note that at about this time the horse was losing some weight and had developed a multiple arthritis. It appeared as if the injections were too large, for after an interval of 3 weeks of no injections and then the injections of smaller doses, the agglutinating titer had again increased by May 14.

In Table 2 are given the results of an experiment which shows that a proper antigen may be treated in various ways without destroying the agglutinability of the streptococci. The agglutinations in the broth culture of the original acidity and after neutralization to phenolphthalein ran quite parallel, differing only slightly from those in the salt solution suspensions of the density of broth culture when the latter were untreated, when they were heated to 60 C. for 30 minutes, when they were heated to 100 C. for 3 minutes and when 0.5% formalin or 0.5% phenol was added. The suspension 15 times the density of the broth culture also yielded comparable results.

In this connection, one point should be emphasized. In Table 1 it may be seen that as the agglutinating titer of the serum increased there was decrease in agglutination in the low dilutions. Instead of agglutination there was often a marked increased cloudiness or precipitation. This observation was made with numerous strains and is being studied in greater detail. Given a strain which was sharply agglutinated in the 1 : 1 and 1 : 10 dilution of normal horse serum, there was often no agglutination or less agglutination in these dilutions of the highly immune serum, but marked agglutination in the higher dilutions (Table 2 and Figs. 3 and 4). If there was no agglutination of certain strains by normal horse serum, the maximum agglutination by the highly immune serum was shifted toward the lower dilutions. Moreover, if the amount of antigen was greatly increased, agglutination tended to be less marked in the low dilutions of normal serum, while in the immune serum in the low dilutions it became more marked (Table 2). It appears, therefore, that no agglutination may be the result of either too little or too much agglutinin, and that antigen and antibody to be effective must be present in definite proportions.

The antigen used in Table 2 was freshly prepared. As previously indicated it was the custom to prepare large amounts of antigen when the condition of the strain was thought to be right, and to preserve

the unused portion in the ice chest. After determining that the agglutinating power of the serum from Horse 1 and Horse 3 on May 14 was comparable to that of previous bleedings, an experiment was performed to test the keeping qualities of various antigens. Twenty-one antigens —17 from human sources, 2 from monkey sources, and 2 controls— were titrated against normal horse serum and the serum of Horse 1 and Horse 3. The interval between the primary agglutination test and this experiment ranged from 50-126 days. To 17 of the antigens, 0.5% phenol was added at the time of preparation. The rest were suspensions in salt solution and still contained living bacteria. The agglutination by the normal horse serum was about the same in the fresh and

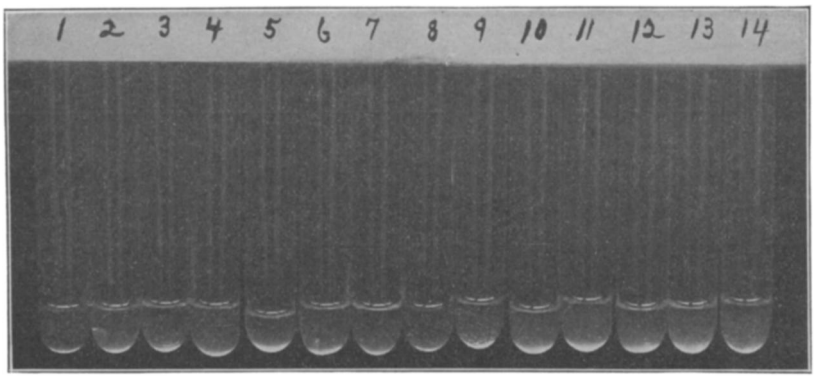


Fig. 4.—Same as Fig. 3 with normal horse serum. Note the complete absence of agglutination of the human strain and slight agglutination in the 1: 1 and 1: 10 dilutions of the monkey strain.

in the preserved antigen. The agglutination by the serum of Horse 1 was not materially changed in 6, was moderately reduced in 7, and markedly less or entirely absent in 6 antigens. The agglutination by the serum from Horse 3 was not materially less than in the original test in 9 instances, moderately less in 5, and markedly less or entirely absent in 5. The loss or maintenance of agglutinability toward the 2 immune serums usually ran parallel. The control antigens showed little or no agglutination. In one instance in which strains from both the tonsil and cord were used the agglutination was markedly reduced in both. Reduction in agglutinability occurred irrespective of whether the bacteria were alive or dead from addition of phenol.

## EXPERIMENTS ON THE AGGLUTINABILITY OF THE STREPTOCOCCUS ISOLATED FROM BRAIN AND CORD OF PARALYZED ANIMALS

The pleomorphic streptococcus was often isolated in large numbers from the brain and cord of animals showing paralytic symptoms when cultures of the blood and other tissues showed few or no organisms. The morphologic and other characteristics of these strains, while quite distinctive when first isolated, were too temporary and sometimes not sufficiently striking to differentiate them unmistakably from strepto-

TABLE 3

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS (714) BEFORE AND AFTER ANIMAL PASSAGE BY THE SERUM OF HORSE 1

| Serum from | Dilutions of Serum | Before Animal Passage | | | | After Animal Passage— | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 714.3 | | | | $714^{3}.7$ | $714^{3}.3$ | | |
| | | Recent Culture, Dried Brain | Old Anaerobic Culture, Fresh Brain | 714.3 Cerebral Fluid | 714.6 Cerebral Fluid | R985 | R998 | R999 | R1000 |
| Normal Horse | 1 : 1 | ++ | + | + | + | + | ++ | + | +++ |
| | 1 : 10 | + | 0 | + | + | ++ | 0 | 0 | + |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | — | — | — |
| | 1 : 31,250 | — | — | 0 | 0 | 0 | — | — | — |
| Horse 1 | 1 : 1 | +c | +c | +++ | 0c | ++ | 0c | 0c | 0c |
| | 1 : 10 | ++ | ++c | +++ | +c | ++ | 0 | + | + |
| | 1 : 50 | ++++ | ++c | +++ | + | ++ | +++ | ++ | ++++ |
| | 1 : 250 | +++ | +++ | +++ | ++ | + | ++ | ++ | ++++ |
| | 1 : 1250 | + | + | ++ | ++ | 0 | + | + | + |
| | 1 : 6250 | + | 0 | + | 0 | 0 | — | — | — |
| | 1 : 31,250 | — | — | + | 0 | 0 | — | — | — |

cocci which produce green colonies on blood agar. The possibility of the occasional presence of other streptococci in small numbers in the brains of these animals was recognized and the need, therefore, for immunologic differentiation was apparent in the early part of the work. At the time of necropsies on paralyzed animals the aspirated brain substance and often also a small amount of ventricular or spinal fluid was filed away in sealed pipets in the refrigerator. It was thought, too, that peculiar properties of these strains might best be maintained when they were kept in this condition. Many of the strains from human poliomyelitis which were used for the immunization of Horse 3 and for the preparation of antigens used in these experiments had been kept in this manner until shortly before performing the experiments. The results of the agglutination experiments with these strains before and after animal passage are now to be given in some detail.

Table 3 shows the agglutinating property of one strain (714) from typical poliomyelitis in a child before and after animal passage, about 5 months after isolation, together with controls. The strain used in the first column had been isolated a short time previously from a piece of the dried brain. The one in the second column was isolated from the fresh brain at the time of necropsy and was preserved in a deep culture of ascites plain tissue broth covered with oil. The strain in the third and fourth columns was isolated from the cerebral fluid at the

TABLE 3—*Continued*

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS (714) BEFORE AND AFTER ANIMAL PASSAGE BY THE SERUM OF HORSE 1

| —After Animal Passage | | | | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $714^4.3$ | $714^5.3$ | | | | | | | | |
| | | | M12 | | $748^2$ | | | | |
| R1017 | R1023 | M10 Brain | Brain | Axillary Lymph Gland | Tonsil R1037 | 756 Teeth | $756^2$ R1039 | 622 | 257 |
| +++ | ++ | +++ | +++ | +++ | + | ++ | 0 | + | 0 |
| + | + | ++ | 0 | ++ | 0 | + | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| — | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — |
| — | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — |
| 0c | +c | 0c | +c | +c | 0 | 0 | 0c | 0 | 0 |
| 0c | ++ | +c | +c | +++ | 0 | 0 | + | 0 | 0 |
| +++ | +++ | ++++ | ++++ | +++ | 0 | 0 | 0 | 0 | 0 |
| ++++ | +++ | +++ | +++ | ++ | 0 | 0 | 0 | 0 | 0 |
| + | ++ | ++ | ++ | 0 | 0 | 0 | 0 | 0 | 0 |
| — | + | + | + | 0 | 0 | 0 | — | — | — |
| — | 0 | + | 0 | 0 | 0 | 0 | — | — | — |

time of necropsy and was preserved in an ascites plain tissue agar stab. The former was in the third subculture, the latter in the sixth. All of these were agglutinated markedly and in high dilutions by the serum from Horse 1, immunized with monkey strains; the one in the sixth culture less markedly than the one in the third. The identification of the strains isolated from animals is so important that the results of the animal experiments are given somewhat in detail.

An emulsion of the fresh brain was injected August 24 into the brain of a young rabbit (978). It developed flaccid paralysis of the left fore leg on the 4th day, and died of respiratory failure. Cultures from the blood of this rabbit were sterile, while those from the brain showed the pleomorphic streptococcus in pure culture. Cultures from the brain in the first and second generation were injected intravenously August 30 into each of 4 young rabbits (985, 998, 999, 1000), the doses ranging from 1 c.c. of culture to the growth from 30 c.c. of culture.

Rabbit 985 developed an ascending flaccid paralysis and died of respiratory failure. Cultures on blood agar plates of the brain substance at the time of necropsy showed enormous numbers of small dry green colonies of the streptococcus. This strain was grown for 6 generations on blood agar and the antigen prepared from the next, or seventh, culture in ascites dextrose broth. It was still in an agglutinable condition as shown in the table.

The strains from Rabbits 998, 999 and 1000, from which the antigens were prepared, had been isolated a short time previously from the brain material which had been preserved in the ice chest for about 6 months. The antigens were prepared from the second dextrose broth culture after one plating on blood agar. Rabbit 998 died 48 hours after injection. Cultures from the blood were sterile, those from the fresh brain showed a large number of green colonies of streptococci, and those from the preserved material showed a few similar colonies. Rabbit 999 developed ataxia, coarse tremor of the head and forelegs on the second day, complete flaccid paralysis of the hind legs and left foreleg on the fourth day, and was found dead on the fifth day. Cultures from the blood were sterile, those from the fresh brain showed 5 typical green producing colonies on blood agar, and the preserved material showed no growth on aerobic blood agar plates and a few green colonies on an anaerobic blood agar slant. Rabbit 1000 was found dead the day after a second injection. Cultures on blood agar plates of the blood were sterile, those of the fresh brain showed 5 green colonies of streptococci, while those from the preserved brain material showed 15 similar colonies. The strains isolated from the fresh and preserved brain of these 3 rabbits appeared identical on blood agar, all producing dry, green colonies of streptococci, which were agglutinated markedly and in high dilutions by the serum from Horse 1.

Rabbit 1017 was injected intravenously September 5 with the growth from 15 c.c. of broth culture from the brain of Rabbit 999. The animal became unsteady and weak on the second day; there was constant tremor of the head and it was just able to stand. The next day it was worse; power in the front legs and left posterior neck muscles was gone and it pushed itself along with the hind legs. It died on the third day. Blood-agar plate cultures from the fresh brain showed innumerable fine green colonies of streptococci, those from the kidney many colonies and those from the blood a few similar colonies. No streptococci were obtained from the liver and joint fluid. The preserved brain material showed countless numbers of green colonies of streptococci. This strain and the strains from the 3 rabbits in the previous animal passage were agglutinated similarly.

Rabbit 1023 was injected intracerebrally September 8 with 0.5 c.c. of a Berkefeld filtrate of the emulsion of the fresh brain of Rabbit 1017; Monkey 10 was injected intracerebrally with 1.5 c.c. of the emulsion; and Monkey 12 was injected intravenously with the growth from 30 c.c. and 60 c.c. of ascites dextrose and plain broth culture from the brain of the same rabbit. Rabbit 1023 remained well for 48 hours. It then developed meningeal and cerebellar symptoms with marked weakness of the adductors of the right hind leg, and died on the third day. Blood-agar plate cultures of the fresh brain and lumbar cord showed countless fine dry green colonies of streptococci in pure culture, while those from the filtrate of the brain and cord emulsion yielded the same organism also in pure culture. The culture from the preserved brain material showed many identical colonies. Cultures from the blood, liver and kidney showed no streptococci.

Monkey 10 developed flaccid paralysis beginning the day after the injection, which extended rapidly until death occurred from respiratory failure. Blood-

agar plate cultures of the fresh brain and cord and of the edematous fluid surrounding the cord showed countless fine dry green colonies of streptococci in pure culture. The cultures from the blood, kidney, spleen and lymph gland were negative. Those from the preserved brain material showed a moderate number of green colonies.

Monkey 12, as previously reported, developed flaccid paralysis beginning in the left arm the day following the injection. This extended and the animal became prostrate on the sevnth day, when it was chloroformed. Cultures from the brain and axillary lymph gland yielded the characteristic organism. Both were preserved in deep cultures until shortly before the agglutination tests were made. This strain in the fourth animal passage from the brain of Rabbit 1023 from the brain of Monkeys 10 and 12 and from the axillary lymph gland of the latter produced green colonies on blood agar at the time of the tests. All were agglutinated markedly and in high dilution as shown in Table 3. The control strain 748 was isolated from the blood of Rabbit 1037, injected with the emulsion of the tonsil from a patient with poliomyelitis 6 weeks after the attack. The rabbit showed no paralysis. The control strain 756, a green producing streptococcus, was isolated from the pyorrheal pockets about the teeth of a monkey with symptoms suggesting poliomyelitis, but sections of the cord showed no lesions. This strain was injected intravenously into Rabbit 1039. No paralytic symptoms were noted. The strain not passed through animals was cultivated on blood agar while the strain after animal passage was preserved in the brain material of Rabbit 1039 and isolated a short time previous to the experiment. The control strain 622 was a pneumococcus and the control strain 257 a hemolytic streptococcus. None of the control strains were agglutinated. Results similar to those given in Table 3 were obtained in numerous instances with other antigens prepared from subsequent cultures of the preserved brain of these animals, and, as will be seen, the condition of many of these strains was such that agglutination took place with the serum of patients and monkeys which had recovered from attacks of poliomyelitis.

According to the agglutination tests the specific organism was isolated in this case of human poliomyelitis, (1) from the fresh brain by direct culture and by the injection of an emulsion into the brain of a rabbit, (2) from the dried brain, months later, and (3) from the cerebral fluid at the time of necropsy. It was possible to recover the organism in the second animal passage from the brain of each of 4 rabbits; in the third animal passage, from the brain of 1 rabbit injected with the filtrate of the brain emulsion of one of these; in the fourth animal passage, from the brain of 1 rabbit and 1 monkey, and from the brain and the axillary lymph gland of another monkey. The organism in all of these 7 animals tended to localize electively in the central nervous system.

Table 4 shows the agglutinating power of immune serum from Horse 1 and Horse 3 over another strain (722) from human poliomyelitis before and after animal passage. All of the strains which were agglutinated produced fine dry green colonies on blood-agar plates

TABLE 4

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS (722) BEFORE AND AFTER ANIMAL PASSAGE BY THE SERUM FROM HORSE 1 AND HORSE 3

| Serum from | Dilutions of Serum | Before Animal Passage | | After Animal Passage | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 722.7 Brain | 722.8 Brain | $722^2.2$ (Brain) R1003 Green-producing Streptococcus | $722^2.3$ (Brain) R1003 Indifferent Streptococcus | $722^2.3$ (Brain) R1015 Hemolyzing Streptococcus | $722^2.4$ (Tonsil) P414 | $722^2.4$ (Cord) P420 Hemolytic Streptococcus | $722^3.3$ (Brain) R1010 |
| Normal Horse | 1 : 1 | ++ | ++ | +++ | 0 | + | 0 | + | ++ |
| | 1 : 10 | + | + | ++ | 0 | 0 | 0 | 0 | ++ |
| | 1 : 100 | + | 0 | + | 0 | 0 | 0 | 0 | 0 |
| | 1 : 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 100,000 | 0 | 0 | — | — | 0 | 0 | 0 | 0 |
| | 1 : 1,000,000 | 0 | 0 | — | — | 0 | 0 | 0 | 0 |
| Horse 1 | 1 : 1 | +c | ++ | + | 0 | + | +++ | +c | +++ |
| | 1 : 10 | + | ++ | + | 0 | 0 | ++ | 0 | ++++ |
| | 1 : 100 | ++ | +++ | ++ | 0 | 0 | + | 0 | ++++ |
| | 1 : 1000 | + | +++ | ++ | 0 | 0 | 0 | 0 | + |
| | 1 : 10,000 | 0 | + | + | 0 | 0 | 0 | 0 | 0 |
| | 1 : 100,000 | 0 | 0 | — | — | 0 | 0 | 0 | 0 |
| | 1 : 1,000,000 | 0 | 0 | — | — | 0 | 0 | 0 | 0 |
| Horse 3 | 1 : 1 | ++ | +++ | | | | ++++ | | +++ |
| | 1 : 10 | +++ | +++ | | | | ++ | | ++++ |
| | 1 : 100 | ++ | +++ | | | | + | | ++++ |
| | 1 : 1000 | 0 | + | | | | 0 | | ++ |
| | 1 : 10,000 | 0 | 0 | | | | 0 | | 0 |
| | 1 : 100,000 | 0 | 0 | | | | 0 | | 0 |
| | 1 : 1,000,000 | 0 | 0 | | | | 0 | | 0 |

at the time the tests were made, just as they did when first isolated. The strain in the seventh and eighth culture which had not been passed through animals had been grown in ascites tissue agar stabs and on blood-agar slants from Sept. 2, 1916, until March 21, 1917. Results similar to those shown in the table were obtained with 4 other antigens from this strain. The strain after 1 or 2 animal passages was preserved in the brain substance placed in sealed pipets in the ice chest until shortly before the tests were made. This material was then plated on blood agar, planted in dextrose or ascites dextrose broth and used for the agglutination tests in the second, third, or fourth culture generation.

Rabbit 1003, 470 gm., was injected intracerebrally Sept. 1, 1916, with 0.25 c.c. of the emulsion of the brain of a patient with typical poliomyelitis (Case 722). The animal died within 24 hours in convulsions, after marked antemortem weakness of the hind extremities. Cultures of the blood were sterile; cultures from the fresh brain yielded a pure growth of fine dry green colonies on blood agar; while those from the preserved material showed 2 types of colonies—a moderate number of fine dry green, and a few indifferent colonies of streptococci. The former type was agglutinated in high dilution by the serum from Horse 1, the latter not at all.

Rabbit 1015, 250 gm., was injected intravenously Sept. 5, 1916, with a scant growth from 30 c.c. of ascites dextrose tissue broth of the streptococcus (in the third generation) from the brain (Case 722). The animal died within 24 hours. There was hemorrhagic edema surrounding the cord and marked softening of the brain and cord. A few small embolic foci were found in the right ventricle and a number of small hemorrhages in the lungs. Cultures of joint fluid, blood, liver, kidney and spleen remained sterile, while the brain and lumbar cord showed countless fine dry green colonies. The preserved brain material showed very many slightly hemolyzing colonies of streptococci distinctly different from those isolated at the time of necropsy. The third culture generation of this strain as shown in Table 4 was not agglutinated by the serum from Horse 1.

Guinea-pig 414 was injected intracerebrally Sept. 1, 1916, with 0.25 c.c. of a salt solution emulsion of the tonsil (Case 722). The animal showed marked weakness, tremor and irritability, and died on the sixth day. Marked extradural hemorrhage in the cervical region of the cord, and a bronchopneumonia were found. There were no lesions of joints or nerves. Cultures from the blood showed 5, from the brain enormous numbers, and from the cord a few fine dry green colonies. The cultures from the lung and liver showed colon bacilli. Blood-agar plate cultures from the preserved brain material remained sterile, but the ascites dextrose broth culture showed the streptococcus and colon bacillus. Plating of this culture showed green colonies of the streptococcus. The agglutination of this strain in the fourth culture was marked in both immune serums although not in dilution over 1 : 100.

Guinea-pig 420 was injected intravenously Sept. 2, 1916, with the growth from 10 c.c. of the primary ascites dextrose broth culture of the cord. Plate cultures of the emulsion injected showed colon bacilli and typical hemolytic streptococci. The animal died in 7 hours. Cultures of the blood and brain showed hemolytic streptococci and colon bacilli; those from the preserved brain material also showed hemolytic streptococci as did the antigen used for the agglutination tests. This strain, although kept under exactly the same conditions as the characteristic streptococcus, was not agglutinated.

Rabbit 1010 was injected intravenously Sept. 3, 1916, with 4 c.c. of ascites dextrose-broth culture from the brain of Rabbit 1003. The animal showed flaccid paralysis of the left front leg, and died in convulsions on the third day. There was found marked edema surrounding the dura of the cord, turbid cerebrospinal fluid and slight clouding of the pia. There were no other lesions. Blood-agar plate cultures of the blood remained sterile, while those of the lumbar cord and brain showed a great many green colonies of streptococci, and those from the preserved brain material showed countless numbers of small dry green colonies of streptococci. The agglutination of this strain was marked, and in dilutions up to 1 : 1000.

Results similar to those given in Table 4 were obtained with the green-producing streptococcus from Rabbit 1003 in 3 other experiments, from Pig 414 in 4, from Rabbit 1010 in 14, and from Rabbit 1015 in 2. Agglutination with antipneumococcus serum, Types I and II, of the strains from Rabbits 1003 and 1010, was no higher than with normal horse serum. The strain from Rabbit 1010 was agglutinated by the serum from a case of sporadic poliomyelitis and by the serum from 11 out of 12 cases of epidemic poliomyelitis, but not by the serum from 5 normal persons. The serum of 7 of 12 paralyzed monkeys agglutinated this strain more markedly or in higher dilution, or both, than the serum of 5 normal monkeys used as controls.

Therefore, according to the agglutination tests, the specific strain in this case was isolated from the tonsil by the injection of an emulsion into the brain of a guinea-pig, and from the brain by direct culture and by the injection of an emulsion into the brain of a rabbit and moreover it was re-isolated in 2 successive animal passages.

Judging from the results of the cultures of the brain of Rabbit 1003, the strain producing indifferent colonies on blood agar which was not agglutinated, should be looked on as having lost the characteristic properties, although the possibility of its being a contaminant must be admitted. The strain from Rabbit 1015 undoubtedly acquired hemolyzing power and accordingly lost its agglutinability. The strain from the cord injected into Pig 420 was clearly a contamination.

According to the agglutination tests, the specific streptococcus was isolated from the tonsil, from the brain, and from the cord (Case 721). The strain from the cord was re-isolated in pure culture from the brain of both a monkey and a guinea-pig that had shown paralysis following intravenous injection 13 and 12 days previously. Similar results were obtained from other cases.

To summarize: According to the agglutination tests, the specific organism was isolated from the fresh or preserved brain material in all of 18 animals (13 rabbits, 2 guinea-pigs and 3 monkeys) injected with strains from 8 cases of poliomyelitis. There was a marked tendency for the organism to localize in the central nervous system. The blood from these animals was sterile in all but 4 instances, and in these it contained only a few of the organisms while cultures from the

TABLE 5

AGGLUTINATION OF STREPTOCOCCI FROM MONKEY 85

| Strain | Dilutions of Serum | Serum from | | |
|---|---|---|---|---|
| | | Normal Horse | Horse 1 (Jan. 30) Immunized with Monkey Strains | Monkey 85 Paralyzed with Virus |
| Green streptococcus from nasal mucous membrane | 1 : 1 | 0 | 0 | 0 |
| | 1 : 10 | 0 | 0 | 0 |
| | 1 : 50 | 0 | 0 | 0 |
| | 1 : 250 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 |
| Pleomorphic streptococcus from cyst in brain | 1 : 1 | 0 | +++ | + |
| | 1 : 10 | 0 | +++ | ++ |
| | 1 : 50 | 0 | ++ | 0 |
| | 1 : 250 | 0 | + | 0 |
| | 1 : 1250 | 0 | 0 | 0 |
| Pleomorphic streptococcus from cord | 1 : 1 | + | ++ | — |
| | 1 : 10 | + | ++ | — |
| | 1 : 50 | 0 | ++++ | — |
| | 1 : 250 | 0 | +++ | — |
| | 1 : 1250 | 0 | ++ | — |
| | 1 : 6250 | 0 | + | — |

brain showed large numbers. Thirteen animals showed symptoms referable to the nervous system and all showed gross or microscopic lesions. Emulsions or cultures from tonsils were injected in 4 instances, emulsions of the brain in 3 and cultures from the brain or cord in the rest.

In Table 5 is given a striking example of the specific agglutinating property of the plemorphic streptococcus isolated from the central nervous system of a monkey paralyzed with virus. The green-producing streptococcus isolated from the nasal mucous membrane was not agglutinated either by the serum of Horse 1 or by the serum of the monkey, whereas the pleomorphic streptococcus from the cyst in the brain which also produced green on blood agar, was markedly agglutinated by the serum of Horse 1 and moderately by the serum from the monkey. The strain from the cord also showed marked agglutination with the serum from Horse 1.

TABLE 6

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS FROM HUMAN POLIOMYELITIS WITH THE SERUM FROM NORMAL HORSE, HORSE 1, AND HORSE 3, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum from | | |
|---|---|---|---|
| | Normal Horse, Percentage | Horse 1, Percentage | Horse 3, Percentage |
| 1 : 1 | 27 | 3 | 5 |
| 1 : 10 | 54 | 14 | 13 |
| 1 : 100 | 8 | 20 | 44 |
| 1 : 1000 | 0 | 40 | 22 |
| 1 : 10,000 | 0 | 16 | 11 |
| 1 : 100,000 | 0 | 2 | 4 |
| 1 : 1,000,000 | 0 | 0.5 | 1 |
| No agglutination | 11 | 8 | 0 |

The upper limit of agglutination was found to be a rough index of the agglutinating power of these serums. In Table 6 is given the total incidence of the upper limit of agglutination of the pleomorphic streptococcus isolated from human poliomyelitis with the serum from Normal Horse, Horse 1, and Horse 3, and the total incidence of no agglutination. The results are given in percentage of incidence; hence are directly comparable to those in other tables and need not be discussed in detail. The need of making many tests with the pleomorphic streptococcus and with many control strains was recognized.

Altogether 211 experiments were made testing the agglutinating power of serum from a normal horse and Horse 1 over 92 different antigens prepared from 19 human poliomyelitis strains; 79 of these

TABLE 7

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF STRAINS FROM MONKEY POLIOMYELITIS WITH THE SERUM FROM NORMAL HORSE, HORSE 1, AND HORSE 3, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum from | | |
|---|---|---|---|
| | Normal Horse, Percentage | Horse 1, Percentage | Horse 3, Percentage |
| 1 : 1 | 24 | 7 | 11 |
| 1 : 10 | 39 | 8 | 14 |
| 1 : 100 | 19 | 28 | 32 |
| 1 : 1000 | 0 | 22 | 21 |
| 1 : 10,000 | 0 | 20 | 11 |
| 1 : 100,000 | 0 | 5 | 4 |
| 1 : 1,000,000 | 0 | 5 | 0 |
| No agglutination | 18 | 4 | 1 |

TABLE 8

AGGLUTINATION OF CONTROL STRAINS OF STREPTOCOCCI AND PNEUMOCOCCI BY THE SERUM OF NORMAL HORSE AND HORSE 1

| Serum | Dilutions of Serum | Strain and Source | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 999 Cholecystitis | 140 Cholecystitis | 341 Parotitis | 773 Ulcer of Stomach | 93 Appendicitis | 130 Myositis | 602 Myositis | 1.70 Pneumococcus Type I |
| Normal Horse | 1:1 | + | +++ | ++ | ++ | 0 | + | + | + |
| | 1:10 | 0 | ++ | + | + | 0 | 0 | 0 | 0 |
| | 1:100 | 0 | + | 0 | + | 0 | 0 | 0 | 0 |
| | 1:1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:1,000,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Immune Horse 1 | 1:1 | 0c | 0c | 0c | 0c | 0c | 0c | 0c | + |
| | 1:10 | 0 | 0c | + | 0 | 0 | 0 | c | + |
| | 1:100 | 0 | + | + | 0 | 0 | 0 | 0 | 0 |
| | 1:1000 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:1,000,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

tests included the serum from Horse 3. Forty of the antigens were prepared from 15 strains isolated from brain and cord before animal passage; 30 from 4 strains after animal passage; 15 from 6 strains from tonsils before animal passage; and 7 from 4 strains from tonsils after animal passage. In 6 instances, antigens from the tonsil and brain or cord of the same case were tested. The upper limit of agglutination of these strains with normal horse serum was 1:1 or 1:10 in nearly all instances. In only 8% of the tests was it 1:100, and in none was it above this point. The upper limit of agglutination with the immune serum from Horse 1 was 1:1000 or above in nearly 60% of the tests,

and with the immune serum from Horse 3 it was 1:1000 in nearly 40% of the tests. In the instances in which no agglutination occurred in the immune serum or in which it was no higher than in the corresponding normal serum, the antigen used had usually been prepared from strains after long cultivation, or after changes in the organism were evident, so that marked agglutination was not to be expected.

In Table 7 is given the total incidence of the upper limit of agglutination of strains from monkey poliomyelitis with the normal and antipoliomyelitic horse serums. Altogether 74 agglutination tests were made with normal horse serum and the serum from Horse 1, 28 of these including the serum from Horse 3. Thirty-nine antigens were used. These were prepared from strains isolated from the central ner-

TABLE 8—*Continued*

AGGLUTINATION OF CONTROL STRAINS OF STREPTOCOCCI AND PNEUMOCOCCI BY THE SERUM OF NORMAL HORSE AND HORSE 1

| Strain and Source | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 622 Pneumococcus Type II | x11[2] Hemolytic Streptococcus | 292 Endocarditis | 276 Herpes Zoster | 281[2] Herpes Zoster | 848 Tonsil | M74 Colitis | B. from Endocarditis | | 864 Tooth |
| | | | | | | | Before Animal Passage | After Four Animal Passages | |
| +++ | + | ++ | ++ | +++ | + | 0 | + | + | 0 |
| + | 0 | + | 0 | + | + | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| +c | +c | ++ | ++ | +c | ++c | 0 | 0 | ++c | 0c |
| 0c | 0 | ++ | 0 | ++ | 0 | 0 | 0 | ++ | 0c |
| 0 | 0 | 0 | 0 | +++ | 0 | 0 | 0 | ++ | + |
| 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

vous system of 20 monkeys that had been paralyzed with virus and 8 monkeys that had received injections of cultures of the pleomorphic streptococcus. The upper limit of agglutination of these strains corresponds quite closely to that of the strains from human poliomyelitis. In the instances in which no agglutination occurred in the immune serum or in which it was no higher than in the normal serum, the antigen had usually been prepared from strains after long cultivation, or after changes in the organism had become evident.

In Table 8 are given the results of agglutination tests of control strains with the serum from Normal Horse and Horse 1. These are

quite representative of the results obtained with a larger series, and include 2 strains (281[3] and B[4]), isolated from cases of herpes zoster and endocarditis, respectively, which were agglutinated in dilutions as high as 1:1000 by the serum from Horse 1. Both had been cultivated for a long time after animal passage. The strain from endocarditis which had not been passed through animals was not agglutinated.

The need for testing the agglutinability of many strains of streptococci from a wide range of sources with these serums was apparent. Pneumococci of Types I and II according to Cole, hemolytic streptococci and Streptococcus mucosus were included in the control series. Altogether 127 agglutination experiments with 103 control strains of streptococci were made with the serum from Normal Horse and from

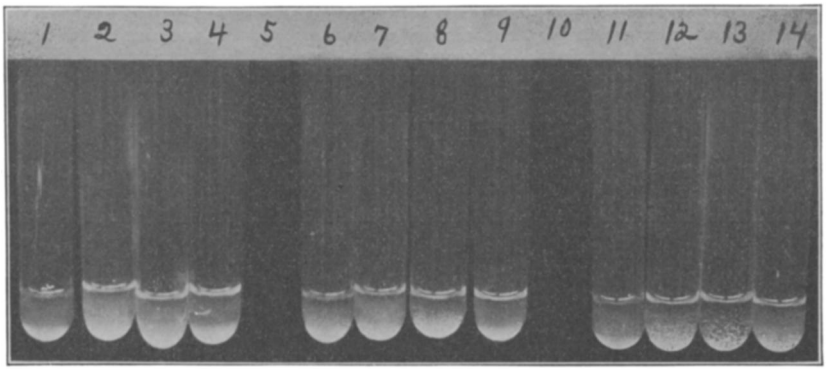


Fig. 5.—Agglutination of a strain from the tonsil in human poliomyelitis by the serum of Horse 1 and 3. Tubes 1-4 contained normal horse serum, 6-9 immune serums Horse 1 and 11-14 immune serums Horse 3. Note absence of agglutination in normal horse serum. Increasing agglutination up to 1:100 in the immune serums and its absence in the 1:1000 dilution.

Horse 1; 12 of these tests included the serum from Horse 3. The strains were isolated from a wide range of diseases or conditions, including 2 strains from meningitis. The length of time since the isolation of the different strains varied a great deal. Some were kept under the same conditions as the poliomyelitis strains from the time they were isolated until they were used in the experiment. Most of the strains had been kept on blood agar.

Table 9 gives the incidence of the upper limit of agglutination and the incidence of no agglutination of these control strains with the serum from Normal Horse, Horse 1, and Horse 3. The agglutinating power of the immune horse serum toward these strains is only slightly higher

TABLE 9

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF CONTROL STRAINS WITH THE SERUM FROM NORMAL HORSE, HORSE 1, AND HORSE 3, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum from | | |
|---|---|---|---|
| | Normal Horse, Percentage | Horse 1, Percentage | Horse 3, Percentage |
| 1 : 1 | 40 | 22 | 17 |
| 1 : 10 | 35 | 24 | 42 |
| 1 : 100 | 3 | 16 | 17 |
| 1 : 1000 | 0 | 9 | 0 |
| 1 : 10,000 | 0 | 2 | 0 |
| 1 : 100,000 | 0 | 0 | 0 |
| 1 : 1,000,000 | 0 | 0 | 0 |
| No agglutination | 20 | 28 | 25 |

than that of the normal horse serum, which is in sharp contrast to the results obtained with the pleomorphic streptococcus. The few strains which were agglutinated in high dilutions by the immune serum from Horse 1 were usually agglutinated by normal horse serum in low dilutions.

These experiments were controlled in still another way. The agglutinating power of serum from horses immunized with pneumococci Types I and II, and of antistreptococcus and antimeningococcus serum was tested.* In Table 10 are given the results of a number of experiments made at the same time in which there were tested the agglutinating power of the antipoliomyelitis serums, antipneumococcus serums Types I and II, antimeningococcus and antistreptococcus serums, over the pleomorphic streptococcus and over control strains. Six antigens from human strains and 2 from monkey strains, together with 2 controls—a pneumococcus Type I and a hemolytic streptococcus (257)—were used. The strains from human poliomyelitis were agglutinated specifically by the serum from Horse 1 and Horse 3. It is of interest to note that after the 2 human strains (722 and 714) had been passed through animals, they became less agglutinable by the serum from Horse 1 and Horse 3 and specifically more agglutinable to antipneumococcus serum Type II. The amount of agglutination with the latter, however, was not as great as with the antipoliomyelitis serums.

The results with the monkey strains (Monkey 126.3, from the cord, and Monkey 126.4, from the brain) are of interest (Table 10). Both produced green colonies on blood agar. Monkey 126 was paralyzed

* We wish here to express our appreciation to Dr. Rufus I. Cole of the Hospital of the Rockefeller Institute and to R. W. Showalter of Eli Lilly and Company for furnishing us with the respective serums used in these experiments.

TABLE 10

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS AND CONTROL STRAINS BY ANTIPOLIOMYELITIS, ANTIPNEUMCOCCUS, ANTIMENINGOCOCCUS AND ANTISTREPTOCOCCUS SERUM

| Strain | Dilutions of Serum | Serum | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Normal Horse | Antipoliomyelitis | | Antipneumococcus | | Anti-meningo-coccus | Anti-strepto-coccus |
| | | | Horse 1 | Horse 3 | Type I | Type II | | |
| 722.8 (Brain) | 1:1 | + | +++ | +++ | +++ | +++ | ++ | +++ |
| | 1:10 | + | ++++ | ++++ | ++ | +++ | + | ++ |
| | 1:100 | 0 | ++++ | ++++ | + | ++ | 0 | ++ |
| | 1:1000 | 0 | + | + | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | + | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | + | | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| 722[3].3 (Brain) (R1010) | 1:1 | ++ | +++ | ++++ | ++ | +++ | ++ | +++ |
| | 1:10 | ++ | +++ | ++++ | + | ++++ | + | +++ |
| | 1:100 | 0 | ++++ | ++++ | 0 | +++ | 0 | + |
| | 1:1000 | 0 | ++ | ++ | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| 714.3 (Brain) | 1:1 | +++ | ++c | +++ | ++ | +++ | | |
| | 1:10 | ++ | ++ | ++++ | ++ | ++ | | |
| | 1:100 | 0 | ++ | ++++ | 0 | 0 | | |
| | 1:1000 | 0 | + | ++ | 0 | 0 | | |
| | 1:10,000 | 0 | + | + | 0 | 0 | | |
| | 1:100,000 | 0 | 0 | 0 | | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| 714[5].2 (Brain) (M10) | 1:1 | +++ | ++ | +++ | +++ | +++ | | |
| | 1:10 | +++ | ++ | +++ | ++ | +++ | | |
| | 1:100 | 0 | ++++ | +++ | 0 | ++ | | |
| | 1:1000 | 0 | ++ | 0 | 0 | 0 | | |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:100,000 | 0 | 0 | 0 | | | | |
| | 1:1,000,000 | 0 | | | | | | |
| 729 (Tonsil pus) | 1:1 | + | ++++ | +++ | + | + | + | + |
| | 1:10 | + | +++ | ++ | 0 | ++ | 0 | 0 |
| | 1:100 | 0 | ++ | 0 | 0 | 0 | 0 | 0 |
| | 1:1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| 734 (Tonsil pus) | 1:1 | ++ | ++c | +++ | ++ | ++ | ++ | + |
| | 1:10 | 0 | +++ | +++ | ++ | + | ++ | ++ |
| | 1:100 | 0 | +++ | ++ | 0 | + | 0 | ++ |
| | 1:1000 | 0 | ++ | + | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | 0 | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| M126.3 (Cord) | 1:1 | ++ | + | + | | | | |
| | 1:10 | + | ++ | +++ | | | | |
| | 1:100 | 0 | +++ | +++ | | | | |
| | 1:1000 | 0 | 0 | 0 | | | | |
| | 1:10,000 | 0 | 0 | 0 | | | | |
| | 1:100,000 | | 0 | 0 | | | | |
| M126.4 (Brain) Green-producing streptococcus | 1:1 | + | + | + | + | 0 | | |
| | 1:10 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:100 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:1000 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:100,000 | 0 | 0 | 0 | 0 | 0 | | |
| | 1:1,000,000 | | | | | | | |
| Hemolytic streptococcus 257 | 1:1 | 0 | + | +++ | 0 | ++ | 0 | +++ |
| | 1:10 | 0 | + | 0 | 0 | 0 | 0 | 0 |
| | 1:100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:10,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | | | | |
| | 1:1,000,000 | 0 | 0 | 0 | | | | |
| Pneumococcus Type I | 1:1 | 0 | 0 | | 0 | + | | |
| | 1:10 | 0 | 0 | | — | — | | |
| | 1:100 | 0 | 0 | | +++ | 0 | | |
| | 1:1000 | 0 | 0 | | + | 0 | | |
| | 1:10,000 | 0 | 0 | | 0 | 0 | | |
| | 1:100,000 | | | | | | | |
| | 1:1,000,000 | | | | | | | |

with virus. At necropsy some time after death, in addition to the typical findings of poliomyelitis, marked ulcerative colitis was found. Besides the green-producing strains a hemolytic streptococcus and colon bacillus were isolated from the nervous system. The green-producing strain from the cord as shown was agglutinated in Table 10, while the one from the brain was not. The former showed short chains, diplococci and single cocci of varying size in fluid cultures and should be regarded as the specific streptococcus. The latter showed long chains of elongated diplococci with no small forms and should be regarded as an accidental invader. The possibility, however, that the latter lost the specific agglutinating property must be considered.

TABLE 11

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF POLIOMYELITIS STRAINS (HUMAN AND MONKEY) BY SERUM FROM HORSES IMMUNIZED WITH PNEUMOCOCCI, MENINGOCOCCI, AND STREPTOCOCCI, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum |
|---|---|
| | Antipneumococcus Antimeningococcus Antistreptococcus |
| | Percentage |
| 1 : 1 | 19 |
| 1 : 10 | 38 |
| 1 : 100 | 32 |
| 1 : 1000 | 0 |
| 1 : 10,000 | 1 |
| No agglutination | 9 |

In Table 11 is given the incidence of the upper limit of agglutination and of no agglutination of the poliomyelitis strains with antipneumococcus, antimeningococcus and antistreptococcus horse serum. Altogether 77 agglutination tests were made with strains from human and monkey sources. A comparison of the figures in Table 11 with the figures in Tables 6 and 7 giving the results of the agglutination of these strains with normal horse serum shows that the agglutinating power of these serums was only slightly higher than that of normal horse serum.

In Table 12 is shown the agglutinating power of normal and immune horse serum and human and monkey serum over a few strains of the pleomorphic streptococcus. Strain 714.3 was isolated a short time previous to this experiment from the dried brain of a patient with typical poliomyelitis. Strain $714^{3}$.3 was isolated from the preserved brain substance of Rabbit 1010, being in the third animal passage. Strain

TABLE 12

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS BY NORMAL AND IMMUNE HORSE, HUMAN, AND MONKEY SERUM

| Strain | Dilutions of Serum | Horse Serum | | Human Serum | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal | Immune Horse 1 | Normal E | Immune 833 | Normal F | Immune 834 | Normal D | Immune 831 |
| 714.3 (Brain) | 1:1 | +++ | ++c | 0 | + | 0 | ++ | ++ | ++ |
| | 1:10 | + | +++ | 0 | ++ | 0 | ++ | + | ++ |
| | 1:100 | 0 | ++++ | 0 | + | 0 | 0 | 0 | ++ |
| | 1:1000 | 0 | ++++ | 0 | 0 | 0 | 0 | 0 | + |
| | 1:10,000 | 0 | ++++ | 0 | + | 0 | + | 0 | + |
| | 1:100,000 | 0 | +++ | 0 | ++ | 0 | 0 | 0 | 0 |
| | 1:1,000,000 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| 714[3].3 (Brain) (R1000) | 1:1 | +++ | ++c | ++ | ++c | ++ | ++c | | |
| | 1:10 | ++ | +++ | 0 | +c | + | ++c | | |
| | 1:100 | 0 | ++++ | 0 | ++ | 0 | + | | |
| | 1:1000 | 0 | ++++ | 0 | ++ | 0 | + | | |
| | 1:10,000 | 0 | +++ | 0 | ++ | 0 | + | | |
| | 1:100,000 | 0 | + | 0 | + | 0 | + | | |
| | 1:1,000,000 | 0 | + | 0 | 0 | 0 | 0 | | |
| 707[3].2 (Cord) (P339) | 1:1 | ++ | +++ | + | ++c | 0 | +c | 0 | +c |
| | 1:10 | ++ | ++ | 0 | ++c | 0 | +c | 0 | ++ |
| | 1:100 | 0 | ++ | 0 | + | 0 | 0 | 0 | ++ |
| | 1:1000 | 0 | +++ | 0 | + | 0 | 0 | 0 | + |
| | 1:10,000 | 0 | +++ | 0 | 0 | 0 | 0 | 0 | + |
| | 1:100,000 | 0 | ++ | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1:1,000,000 | 0 | ++ | 0 | 0 | 0 | 0 | 0 | 0 |
| M97.2 (Brain) | 1:1 | +++ | +++ | + | +++ | + | ++ | + | +c |
| | 1:10 | ++ | ++++ | + | ++ | 0 | ++ | 0 | 0 |
| | 1:100 | 0 | ++++ | 0 | ++ | 0 | + | 0 | + |
| | 1:1000 | 0 | ++ | 0 | 0 | 0 | ++ | 0 | + |
| | 1:10,000 | 0 | + | 0 | + | 0 | ++ | 0 | 0 |
| | 1:100,000 | 0 | 0 | 0 | + | 0 | ++ | 0 | 0 |
| | 1:1,000,000 | 0 | 0 | | | 0 | 0 | 0 | 0 |

Monkey Serum

| Normal 1 | Immune M52 | Normal M121 | Immune M24 | Normal 3 | Immune M85 | Normal 1 | Immune M97 |
|---|---|---|---|---|---|---|---|
| + | ++++ | 0 | ++ | | | | |
| 0 | 0 | 0 | +++ | | | | |
| 0 | + | 0 | ++ | | | | |
| 0 | 0 | 0 | + | | | | |
| 0 | 0 | 0 | 0 | | | | |
| + | +++ | 0 | +++ | 0 | +++ | + | +++ |
| + | + | 0 | + | 0 | ++ | 0 | ++++ |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | +++ |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | + |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | +++ | | | | | | |
| + | ++ | | | | | | |
| 0 | + | | | | | | |
| 0 | + | | | | | | |
| 0 | + | | | | | | |
| + | +++ | | | 0 | + | 0 | + |
| + | +++ | | | 0 | + | 0 | ++ |
| 0 | 0 | | | 0 | 0 | 0 | ++ |
| 0 | 0 | | | 0 | 0 | 0 | + |
| 0 | 0 | | | 0 | 0 | 0 | 0 |

$707^{3}.2$ was isolated August 19 from the fresh cord of a patient and was preserved in an anaerobic culture after 2 animal passages. The strain, Monkey 97, was recently isolated from the brain of a monkey paralyzed with virus. The results in the latter were similar to those shown in Figure 6.

Judging from the agglutinating power of the normal horse serum over these strains it is evident that they were in a relatively agglutinable condition, and hence favorable for detecting differences in the agglutinin content of normal serum and of the serum of patients and monkeys with poliomyelitis. It will be noted that the agglutination by the serum of normal persons and normal monkeys if present at all is

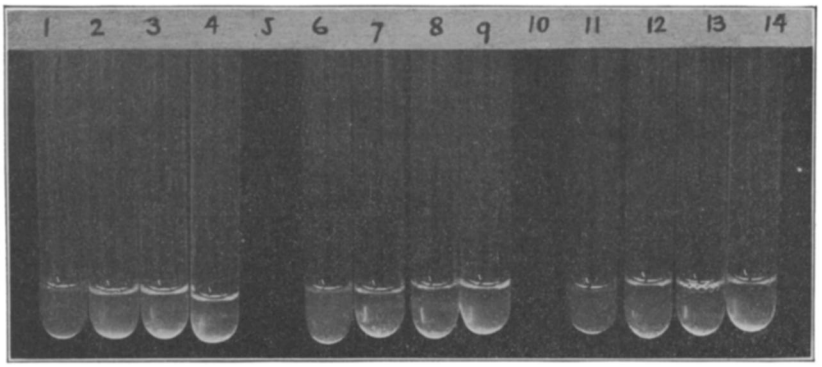


Fig. 6.—Agglutination of monkey strain (M 49.4) with human serum. Tubes 1-4 contained normal human serum. Tubes 6-9 and 11-14 contained the serum from 2 individuals (828 and 846) who had recovered from severe attacks of poliomyelitis. The control serum was obtained on the same day as the immune serums from an individual of approximately the same age. Note the absence of agglutination in the normal serum and the agglutination in the 1: 1 and 1: 10 dilutions of the immune serums.

slight; that it occurs only in low dilutions and is about equal. These serums, however, vary in their agglutinating power over the different strains. The ages of the normal and immune persons in the parallel columns were about the same, and the serums were obtained on the same day. The 3 patients (Cases 833, 834, and 831) were children in New York who had recovered from severe attacks of poliomyelitis with marked residual paralysis.* The agglutinating power of these serums, while not equally great over the different strains, was greater than the corresponding normal control in each instance, and in some instances was evident in high dilutions. The occurrence of increased clouding in

* We are indebted to Dr. George W. Wheeler and Dr. E. D. Ebright of New York, for the serum of these and other cases.

the low dilutions in some of these immune serums is in accord with the observations of the immune horse serums.

Monkey 52 was injected with a culture (in the second generation) of the pleomorphic streptococcus isolated from the brain of a poliomyelitic monkey (Monkey 34). Its serum agglutinated these strains markedly.

Monkey 24 was bled 4 months after it had recovered, with marked residual paralysis, from typical poliomyelitis following injection of virus.

Monkeys 85 and 97 were bled when nearly dead, two days after the onset of typical attacks of poliomyelitis, paralysis beginning six and ten days respectively, after intracerebral injection of virus.

The agglutinating power of the serum of these virus-immune monkeys was greater than the corresponding normal control in each

TABLE 13

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS BY THE SERUM OF NORMAL, VIRUS IMMUNE, AND CULTURE IMMUNE MONKEYS

| Strains | Dilutions of Serum | Serum | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Normal Monkey | | | Monkey 21 | Monkey 24 | Monkey 19 | Monkey 14 |
| | | A | B | C | | | | |
| 714.3 (Brain) | 1 : 1 | 0 | 0 | + | ++ | +++ | ++ | |
| | 1 : 10 | 0 | 0 | 0 | + | +++ | ++ | |
| | 1 : 50 | 0 | 0 | 0 | + | ++ | + | |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | + | |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | |
| M85.3 (Brain) | 1 : 1 | 0 | 0 | 0 | + | +++ | + | |
| | 1 : 10 | + | 0 | 0 | ++ | ++ | ++ | |
| | 1 : 50 | + | 0 | 0 | +++ | ++ | +++ | |
| | 1 : 250 | 0 | 0 | 0 | + | ++ | + | |
| | 1 : 1250 | 0 | 0 | 0 | 0 | + | 0 | |
| Bryan (Control) | 1 : 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 276 (Control) | 1 : 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | |
| $721^2.3$ (Cord) (P444) (Brain) | 1 : 1 | | | 0 | ++ | ++ | + | + |
| | 1 : 10 | | | 0 | ++ | ++ | + | ++ |
| | 1 : 100 | | | 0 | + | ++ | + | + |
| | 1 : 1000 | | | 0 | + | + | 0 | 0 |
| | 1 : 10,000 | | | 0 | 0 | 0 | 0 | 0 |
| M49.4 (Brain) | 1 : 1 | | | ++ | +++ | ++ | + | +++ |
| | 1 : 10 | | | 0 | +++ | +++ | ++ | +++ |
| | 1 : 100 | | | 0 | ++ | ++ | + | 0 |
| | 1 : 1000 | | | 0 | + | + | 0 | 0 |
| | 1 : 10,000 | | | 0 | 0 | 0 | 0 | 0 |

instance. Exactly comparable results are shown in Table 13 with still other strains and serums.

Monkeys 21 and 24 had recovered from attacks of poliomyelitis following injection of virus. Monkey 19 had been rendered resistant to virus by intracerebral injection of cultures of the pleomorphic streptococcus, and Monkey 14 by virus derived from culture (Fig. 7).

The strain, Monkey 85.3, was recently isolated from the brain of a poliomyelitic monkey; the strain, Monkey 49.4, was isolated some time previously from the filtrate of the brain emulsion of a poliomyelitic monkey and was preserved in a deep stab culture. Strain $721^{3}.3$ was

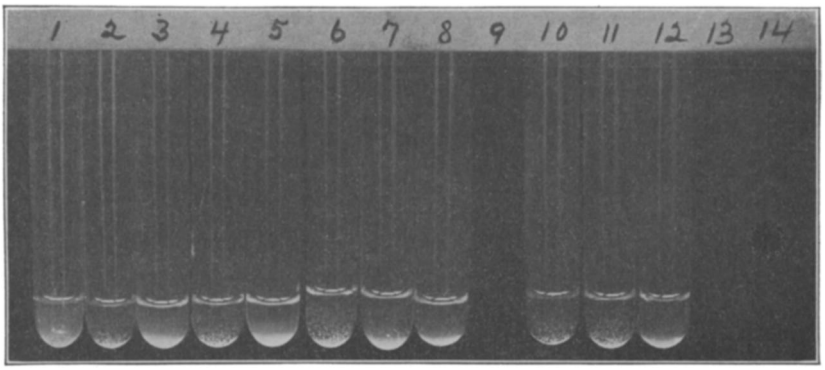


Fig. 7.—Agglutination of the pleomorphic streptococcus from poliomyelitis in the monkey (M. 49.4) with the serum of Monkey 21 paralyzed with virus, and the serum of Monkey 14 which resisted virus following intracerebral injection of virus from culture. Tubes 1, 3, 5 and 7 contained normal monkey serum, Tubes 2, 4, 6 and 8 contained immune serum Monkey 21, and Tubes 10, 11, and 12 contained immune serum Monkey 14. Note the complete absence of agglutination by the normal serum above the first or 1:1 dilution and the marked agglutination by the immune serums. The readings of this experiment are shown in Table 13.

isolated from the cord of a case of human poliomyelitis; it produced paralysis in a guinea-pig and was preserved in the brain of this animal until shortly before the antigen was prepared. The antigen 714.3 was the same as that used in the experiments in Table 2. The strains marked Bryan, and 276, used as controls, were from endocarditis and herpes zoster, respectively. These were not agglutinated by any of the serums. The serum from the virus-immune monkeys agglutinated all the poliomyelitic strains to a greater degree than any of the normal monkey serums. The point of special interest, however, is the fact that the serums from 2 monkeys (19 and 14) rendered resistant to virus by injection of culture of the pleomorphic streptococcus without apparently having had poliomyelitis, agglutinated these strains. In

TABLE 14

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF POLIOMYELITIS STRAINS BY NORMAL AND IMMUNE HUMAN SERUM, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum | |
|---|---|---|
| | Normal, Percentage | Immune, Percentage |
| 1 : 1 | 10 | 12 |
| 1 : 10 | 21 | 29 |
| 1 : 100 | 12 | 23 |
| 1 : 1000 | 0 | 12 |
| 1 : 10,000 | 0 | 5 |
| 1 : 100,000 | 0 | 1 |
| 1 : 1,000,000 | 0 | 1 |
| No agglutination | 57 | 18 |

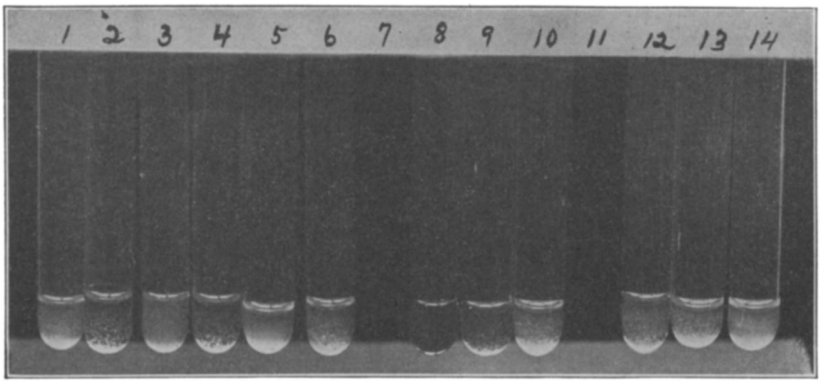


Fig. 8.—Agglutination of the pleomorphic streptococcus from poliomyelitis in the monkey (M 49.4) with the serum of monkeys paralyzed with virus. Tubes 1, 3, and 5 contained normal monkey serum, Tubes 2, 4, and 6 immune serum Monkey 53, Tubes 8, 9, and 10 immune serum Monkey 105, and Tubes 12, 13 and 14 immune serum Monkey 92. The serum of immune Monkeys 53, 105 and 92 was obtained 14, 8 and 16 days, respectively, after onset of severe attacks of poliomyelitis. All were given intravenous injections of serum from Horse 1 after paralysis had begun and all recovered.

addition to these experiments, the serums from Monkeys 21 and 19 together with the serums from 3 normal monkeys were tested against 1 other strain from human poliomyelitis (707) and 1 other strain from monkey poliomyelitis (Monkey 97). Agglutination occurred with the immune serum but not with the normal.

In Table 14 is given the incidence of the upper limit of agglutination of poliomyelitis strains by normal and immune human serum and the total incidence of no agglutination. These results were obtained in experiments in which 27 antigens were tested with the serums from 41 normal persons, from 2 persons having Streptococcus viridans infec-

tion, and from 27 poliomyelitis patients. Eighteen of the antigens were prepared from 13 human strains, and 9 from 4 monkey strains. Altogether there were made 137 agglutination tests with normal human serum and 146 with the serum from patients who had recovered from attacks of poliomyelitis. The upper limit of agglutination in these immune serums does not average as high as in the serums of horses hyperimmunized with these strains, but is sufficiently higher than in the normal controls to be of significance. The incidence of no agglutination was significantly less in the immune than in the normal serum. The agglutinating power of the serums from the 2 patients having Streptococcus viridans infections was no higher than that of some of the

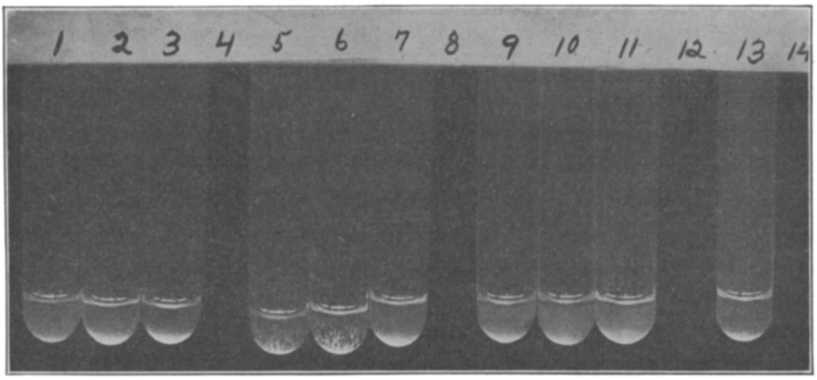


Fig. 9.—Agglutination of human strain after 5 animal passages (714⁸.2) by monkey serum. Tubes 1-3 contained normal monkey serum (151). Note the slight agglutination in the 1: 1 dilution. Tubes 5-7 contained the serum of Monkey 144 paralyzed with virus. Note the marked agglutination in the 1: 1 and 1: 10 dilutions. Tube 13 was the salt solution control. Tubes 9-11 contained the serum of Monkey 144 paralyzed with virus and a suspension of a streptococcus from the brain of a monkey which died with poliomyelitis and ulcerative colitis.

normal serums. The agglutinating power of 4 of the normal human serums over some of these strains was nearly as marked as that of some of the immune serums.

All the normal individuals whose serum was used in these experiments were from regions where poliomyelitis occurred in epidemic form during the summer of 1916; 2 of them had been in close contact with the disease in man and animal. Four of the 25 poliomyelitis patients whose serum was tested were sporadic cases; the others occurred during the epidemic in the summer of 1916. Parallel agglutinations were obtained with the serum from the sporadic and epidemic cases, with the exception of the 1 sporadic case previously reported, the serum from which did not agglutinate these strains.

TABLE 15

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF HUMAN AND MONKEY POLIOMYELITIS STRAINS BY NORMAL AND IMMUNE MONKEY SERUM, AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum from | | |
|---|---|---|---|
| | Normal Monkeys, Percentage | Virus Immune Monkeys, Percentage | Culture Immune Monkeys, Percentage |
| 1 : 1 | 6 | 2 | 7 |
| 1 : 10 | 21 | 33 | 32 |
| 1 : 100 | 9 | 20 | 16 |
| 1 : 1000 | 3 | 16 | 16 |
| 1 : 10,000 | 0 | 5 | 12 |
| No agglutination | 61 | 25 | 18 |

Table 15 gives a summary of the results obtained with the serums from normal and immune monkeys over strains from human and monkey poliomyelitis (Figs. 8 and 9). Altogether 30 antigens were used: eighteen from 18 human strains, 10 before and 8 after, 1-4 animal passages, and 12 from 10 monkey strains before animal passage. The serum from 27 normal monkeys was used in a total of 152 agglutination tests; the serum from 27 virus-immune monkeys in 200 tests; and the serum from 14 monkeys immunized with cultures of the pleomorphic streptococcus or with emulsions of brain or cord of monkeys that had been injected with aerobic cultures in 57 tests.

The results correspond very closely to those obtained with normal and immune human serums. Some of the culture-immune monkeys appeared to have had abortive attacks of poliomyelitis and the serums of 2 had the power to neutralize virus in the test tube.[2]

TABLE 16

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF CONTROL STRAINS BY NORMAL AND IMMUNE HUMAN AND MONKEY SERUM AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

| Dilutions of Serum | Serum | |
|---|---|---|
| | Normal, Percentage | Immune, Percentage |
| 1 : 1 | 26 | 2 |
| 1 : 10 | 11 | 35 |
| 1 : 100 | 18 | 2 |
| 1 : 1000 | 0 | 4 |
| 1 : 10,000 | 0 | 0 |
| 1 : 100,000 | 0 | 0 |
| 1 : 1,000,000 | 0 | 0 |
| No agglutination | 46 | 58 |

In Table 16 is given a summary of the agglutinating power of normal and immune human and monkey serum over control strains of streptococci. In these experiments there were used altogether 18 antigens prepared from 15 strains of streptococci or pneumococci from sources other than poliomyelitis. Thirty-five tests were made with normal human and monkey serum and 52 with immune human and monkey serum. The upper limit of agglutination and the incidence of no agglutination in the normal and immune serums is very nearly alike and corresponds closely to the results obtained with normal human and normal monkey serums over the poliomyelitis strains.

The agglutinating power of the serum from Horse 1 and Horse 3 was tested over 7 strains isolated from the nervous system in poliomyelitis, kindly sent me by Dr. Baldwin Lucke of Philadelphia. These had been cultivated continuously aerobically on ascites dextrose-agar and blood-agar slants since isolated during the summer of 1916. They resembled very closely the strains with which we have worked. Three were of the micrococcus type and 4 of the streptococcus type at the time the antigens for agglutination tests were prepared. Five of the strains were agglutinated markedly and to about the same degree by the serum from Horse 1 and Horse 3; 2 were not agglutinated. The upper limit of agglutination was 1 : 1000 or above in 13 out of a total of 29 tests. In the normal horse serum the upper limit was never above 1 : 100 and usually occurred only in dilutions of 1 : 1 or 1 : 10 if at all. The serum from 2 persons who had recovered from poliomyelitis agglutinated specifically 3 of 6 of these strains.

The results of the agglutination tests with a strain isolated from the pons in human poliomyelitis, kindly sent me by Dr. Meyer Solis-Cohen of Philadelphia, are also of interest. He has found a high opsonic index toward this strain in the serum of patients who have recovered from poliomyelitis. The serum from Horse 1 and Horse 3 agglutinated this strain specifically in high dilutions, on repeated occasions. It was also agglutinated by the serum of 8 virus-immune monkeys.

### SUMMARY OF EXPERIMENTS ON THE AGGLUTINATION OF STRAINS ISOLATED FROM THE TONSIL

It was difficult to isolate the pleomorphic streptococcus from the tonsil and to differentiate it unmistakably from the green-producing streptococcus normally present in tonsils. It was thought worth while therefore to make agglutination tests with strains from tonsils which from their morphologic and cultural characteristics or pathogenic power were considered of etiologic importance when isolated. Altogether 41

agglutination tests with the serum from Horse 1 and Horse 3 were made with 28 antigens prepared from 16 tonsil strains, 20 before animal passage and 8 after 1 or 2 animal passages. Most of the strains had been grown on aerobic blood-agar slants since they were isolated in the summer of 1916 and were in the fourth to the tenth culture generation. Some were kept in deep stabs of ascites tissue fluid and a few in sealed pipets containing the brain substance of paralyzed animals.

Agglutination was more marked with the immune serum of Horse 1 than with the normal horse serum in all but 9 tests. The negative tests included 5 antigens, 4 of which were made from a tonsil strain isolated 6 weeks after the onset of attack and after 1 animal passage. Two tests were made with a strain cultivated aerobically on blood agar since isolation and 2 with strains obtained from the tonsils of 2 cases of sporadic anterior poliomyelitis some weeks after the attack. In these, negative results were to be expected. In only 1 test was the result contrary to expectations. In this instance the antigen was prepared from a strain from the brain of a cat which developed paralysis following intravenous injection of a culture made from the tonsil at the time of the attack.

Agglutination was more marked in the immune serum of Horse 3 than in the serum of Horse 1 in all but 3 tests (Fig. 5). The negative tests occurred with 3 antigens prepared from 3 strains cultivated continuously aerobically on blood-agar slants since isolation. In 2 instances, parallel tests were made with antigens from the same strains, 1 grown continuously on blood agar, the other in deep tissue agar stabs. In one, the upper limit of agglutination was 1 : 1000 by both serums with the antigen prepared from the stab cultures and 1 : 100 by both serums with the antigen prepared from the blood-agar culture. In the other instance the antigens from both types of cultures were agglutinated alike, the upper limit being 1 : 100. No agglutination whatsoever occurred with the normal serum 10 times, whereas only once did the immune serum fail to agglutinate.

Some of the more sensitive strains were selected for agglutination tests with immune human serum and immune monkey serum. Agglutination above that with the respective normal serum was obtained in 5 out of 8 experiments with immune human serum and in 8 out of 14 experiments with immune monkey serum. Immune human and immune monkey serum had no agglutinating power over staphylococci, diphtheroid bacilli and colon bacilli isolated from the nervous system of patients and monkeys.

DEVELOPMENT OF AGGLUTININS IN THE SERUM OF MONKEYS

During the course of these experiments it was found that the agglutinin content of the immune serums from horse, man and monkey did not become appreciably less for some weeks if kept in the ice chest. In most of the experiments the control normal serum was obtained on the same day as the immune serum. In only 2 instances was the serum of the same monkey used as normal and immune. It was desirable therefore to test the agglutinin content of the serum of monkeys previous to injection and at intervals following injection of active virus. If the development of agglutinin has significance, agglutination should occur with strains from human sources and with homologous as well as heterogeneous strains isolated from poliomyelitic monkeys.

In Table 17 is given a summary of an experiment in which these conditions were fulfilled. There were used as antigens 1 strain from brain and tonsil (729), 1 from the pons from a case of typical poliomyelitis in man (899), and 1 from the cord of a monkey (Monkey 145) paralyzed with the virus which we had used in connection with the above experiments (heterogeneous strain), and 1 from a monkey (Monkey 148) paralyzed with the virus obtained recently from the Public Health Laboratories and which was used to inject the monkeys in this experiment (homologous strain). Several control strains were included, the results of only one ($622^{35}.7$) are given in the table. The poliomyelitis antigens were previously found to be agglutinated specifically in high dilution by the serum from the immune horses, and for this reason were selected for the experiment.

Monkeys 147 and 150 (given 1 injection of immune serum on April 21) were first used as controls. All were bled every other day from April 18 to April 29. The blood was allowed to clot, placed in the ice chest, and the serum drawn off the following day. All serums were kept in the ice chest until used.

On April 21, Monkey 148 was injected intracerebrally with 1 c.c. of a 5% emulsion of the glycerinated virus. Paralysis was marked in both arms April 27, and April 29 the animal was completely prostrate. The agglutination experiment with the serum obtained up to April 29 was performed May 10.

On May 2, Monkeys 147 and 150 were bled again and then injected intracerebrally with 1.5 c.c. of a 5% emulsion of the glycerinated virus. Monkey 147 became paralyzed May 9 and was prostrate May 14. The results on the dates given in the table indicate accurately those obtained

TABLE 17

THE DEVELOPMENT OF AGGLUTININS FOR THE PLEOMORPHIC STREPTOCOCCUS IN THE SERUM OF MONKEYS WITH PARALYZED VIRUS

| Strain | Dilutions of Serum | Serum from | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Monkey 147 | | | | Monkey 148 | | | Monkey 150 | | | |
| | | Normal | | | Immune | Normal | | Immune | Normal | | | Immune |
| | | 4/18 | 4/29 | 5/2 | 5/14 | 4/18 | 4/20 | 4/29 | 4/18 | 4/29 | 5/2 | 5/14 |
| 729.9 (Cord) | 1 : 1 | — | — | — | 0 | — | — | — | — | — | — | +++ |
| | 1 : 10 | 0 | 0 | 0 | + | + | + | ++ | + | + | 0 | ++ |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 31,250 | 0 | | | | | | | | | | |
| 729 (Tonsil pus) | 1 : 1 | — | — | — | 0 | — | — | — | — | — | — | + |
| | 1 : 10 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | 0 |
| | 1 : 50 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | 0 | 0 | 0 |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 899 (Pons) | 1 : 1 | — | — | — | ++ | — | — | — | — | — | — | +++ |
| | 1 : 10 | + | 0 | 0 | ++ | ++ | 0 | +++ | + | + | 0 | +++ |
| | 1 : 50 | 0 | 0 | 0 | + | 0 | 0 | ++ | + | + | 0 | ++ |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M145.3 (Cord) | 1 : 1 | — | — | — | 0c | — | — | — | — | — | — | +c |
| | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 | ++ | 0 | 0 | 0 | ++ |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M148.2 (Cord) | 1 : 1 | — | — | — | + | — | — | — | — | — | — | ++ |
| | 1 : 10 | 0 | 0 | 0 | ++ | 0 | 0 | ++ | 0 | 0 | 0 | ++ |
| | 1 : 50 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | + |
| | 1 : 250 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 |
| | 1 : 1250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 : 6250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $622^{35.7}$ (Pneumococcus) Control | 1 : 1 | | | — | 0 | | | | | | — | — |
| | 1 : 10 | | | 0 | 0 | | | | | | 0 | 0 |
| | 1 : 50 | | | 0 | 0 | | | | | | 0 | 0 |
| | 1 : 250 | | | 0 | 0 | | | | | | 0 | 0 |
| | 1 : 1250 | | | 0 | 0 | | | | | | 0 | 0 |
| | 1 : 6250 | | | 0 | 0 | | | | | | 0 | 0 |

in the other bleedings except that in Monkey 148 there was a distinct increased agglutinating power of the serum on April 27 over all the strains. This increase, however, was not as marked as on April 29. There was no demonstrable diminution in the agglutinating power of the serum due to age.

The agglutinin content of the serum was increased in all the monkeys that developed typical attacks of poliomyelitis. This was shown toward all the strains except in 2 instances. The increase in agglutinating power by the immune serum was no greater toward the homologous strain (Monkey 148) than toward the heterogeneous strains (729, 899 and Monkey 145). The serum of Monkey 148, however,

agglutinated the strain from this monkey slightly more markedly than did that of Monkeys 147 and 150. There was no increase in agglutination of the control pneumococcus strain. The cultures from the brain of Monkey 148 showed, in addition to the pleomorphic streptococcus, a hemolytic streptococcus which was not agglutinated by the serum from any of the bleedings of this monkey.

## SUMMARY

The pleomorphic streptococcus isolated from the tonsil and central nervous system of human poliomyelitis and from the central nervous system of monkeys paralyzed with virus has marked antigenic properties.

The strains from both human and monkey poliomyelitis are cross-agglutinated in high dilution by the serum from horses hyperimmunized with human and monkey strains respectively, and in lower dilution by the serum of persons who have had poliomyelitis. Moreover the serum of monkeys acquires specific agglutinating power over these strains as they develop poliomyelitis following injection of virus. This agglutinating power of the serum following poliomyelitic attacks has been shown to persist for months, and hence cannot be regarded as due to mobilization of preformed antibodies or to nonspecific changes in the serum incident to fever etc. at the time of the attack. Streptococci and pneumococci from sources other than poliomyelitis are with few exceptions not agglutinated more by the antipoliomyelitis serums than by normal horse serum. A few of a large number of strains approached in agglutinability the pleomorphic streptococcus.

Normal human and normal monkey serum has little or no agglutinating power over the poliomyelitis strains or over control strains. The agglutinin content toward these strains of serum of persons and monkeys suffering from other streptococcus infections was no higher than of the respective normal serums. Poliomyelitic human and monkey serums showed no increase in agglutinating power over streptococci from sources other than poliomyelitis. Antipneumococcus, antimeningococcus and antistreptococcus horse serums do not agglutinate the poliomyelitic strains more than normal horse serum.

Judging by the results with normal horse serum the pleomorphic streptococcus is more easily agglutinated than green-producing streptococci and pneumococci from a wide range of sources. A method has thus been found which proves that the streptococcus, found so con-

stantly in poliomyelitis, is immunologically quite distinct when first isolated. There is a marked difference in the degree with which the various strains retain their specific agglutinability. Anaerobic cultivation tends to preserve this property; aerobic cultivation tends to destroy it. It may be lost by either method without noticeable changes in morphology or cultural characteristics, but usually these changes occur simultaneously. Some strains retain the specific agglutinating condition through many culture generations. In some instances it may be lost suddenly even during one subculture. The specific agglutinating condition was preserved for months in the dried brain substance of human cases and in brain substance in sealed pipets of animals showing paralysis.

By means of agglutination experiments it has been possible to differentiate the pleomorphic streptococcus from green-producing streptococci isolated occasionally from the central nervous system of uninoculated and inoculated animals. The latter may be regarded as antemortem or postmortem invaders.

The results support the view that the elective localizing power of the pleomorphic streptococcus as first demonstrated by Rosenow, Towne and Wheeler[4] has significance and that it in some way bears etiologic relationship to epidemic poliomyelitis.

1. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: Observations on Immunity of Monkeys to Experimental Poliomyelitis. Jour. Am. Med. Assn., 1917, 68, pp. 280-282.

2. Rosenow, E. C.: The Production of an Antipoliomyelitis Serum in Horses by Inoculations of the Pleomorphic Streptococcus from Poliomyelitis. Jour. Am. Med. Assn., 1917, 69, pp. 261-265.

3. Rosenow, E. C.: The Treatment of Epidemic Poliomyelitis with Immune Horse Serum. Preliminary Report. Jour. Am. Med. Assn., 1917, 69, pp. 1074-1075.

4. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: The Etiology of Epidemic Poliomyelitis. Preliminary Note. Jour. Am. Med. Assn., 1916, 67, pp. 1202-1205.